

Supplemental Materials

Expanded Methods

SARS-CoV-2 RT-PCR testing

COVID-19 molecular testing/confirmation was based on Abbott M2000 or Abbott Alinity m RT-PCR-based detection of the SARS-CoV-2 nucleocapsid (NCP) gene detection in nasopharyngeal swab samples collected in viral transport media.³²

SARS-CoV-2 IgG and IgM serology testing

The brief methodology of the different SARS-CoV-2 serology tests used in this case study are as follows:

IgM_{SP} assay

The FDA-approved AdviseDx SARS-CoV-2 IgM_{SP} assay was performed on the Abbott Alinity i platform per the manufacturer's instructions.³² This semiquantitative chemiluminescent microparticle (CMIA) assay detects IgM antibodies to the spike protein of SARS-CoV-2 in human serum and plasma sample. A vendor-recommended cutoff of 1.0 (index value) for reactivity/positivity of infection (FDA approval nos. for CoV-2 IgM, 6R87 and H14977R01) was applied.

IgG_{SP} assay

The quantitative measurement of IgG_{SP} can be helpful to evaluate an individual's humoral response to vaccines. AdviseDx SARS-CoV-2 IgG II (FDA approval nos. for CoV-2 IgG II, 6S60 and H18575R01)/SARS-CoV-2 IgG II Quant assay (CE marked) were performed on the Abbott Alinity i platform in accordance with the manufacturer's package insert and as described by us previously.³² In this CMIA test, the SARS-CoV-2 antigen-coated paramagnetic microparticles bind to the IgG antibodies that attach to the virus's

spike protein in human serum and plasma sample. The strength of resulting chemiluminescence in relative light units (RLU) following the addition of anti-human IgG labeled in comparison with the IgG II calibrator/standard reflects the quantity of IgG_{SP} present. Fifty arbitrary units per milliliter and above in this test are considered positive.

IgG_{NC} assay

The Abbott Alinity i SARS-CoV-2 anti-nucleocapsid protein IgG semiquantitative CMIA assay has been previously verified for routine patient COVID-19 testing in our institution's clinical laboratory. This assay was performed as described previously³² and the index values of 1.4 and above are considered positive per the manufacturer's instructions.

Profiling of cytokine/chemokine response and antibody response to viral antigens using microarrays

This study used a custom developed fluorescent-based multiplex proteome microarray for the detection of IgG antibodies to 66 different antigens including 18 virus antigens (including SARS-CoV-2 and other coronavirus) and 48 cytokine/chemokine antigens. The profiling was carried out at Microarray and Immune Phenotyping core facility of UT Southwestern Medical Center as described previously.^{33,34} Briefly, DNase-I-pretreated 2 µl serum samples were diluted 1:50 in PBST buffer and incubated with the viral and autoantigens printed in duplicates on nitrocellulose film-coated slides/arrays (Grace Bio-Labs). The binding of antibodies with arrayed antigens was detected using cy3-conjugated anti-human IgG (Jackson ImmunoResearch Laboratory, USA. 1:1000). The images were captured with a Genepix 4200A scanner (Molecular Device) with laser wavelength of 532 nm and 635 nm and analyzed using Genepix Pro 7.0 software

(Molecular Devices). The averaged fluorescent signal intensity of each antigen was subtracted by signal from the spot background and PBS control and normalized to human IgG (internal controls) to obtain the normalized fluorescence intensity (NFI). This served as a quantitative measurement of the binding capacity of each patient sample-derived autoantibodies/antibodies with the corresponding autoantigen or viral antigen analytes. The NFI of each analyte was used to generate heatmaps using Graphpad Prism 10.1 software.

Cytokine analysis

The fluorescent bead-based Bio-Plex Pro Human Cytokine Screening Panel (48-Plex #12007283, Bio-Rad) was used to quantify cytokines in the serum samples of the patient of interest and comparative groups according to the manufacturer's instructions. The results of this panel that includes clinically and biologically relevant assortment of adaptive immunity cytokines, pro-inflammatory cytokines, and anti-inflammatory cytokines (Table 3) were read in a multiplex immunoassay platform (Luminex). An arbitrary value of 0.01 was assigned to the samples that showed a concentration below the detection range. All analytes were expressed as pg/mL and extrapolated from the standard curves (individual for each cytokine).

Serum autoantibody profiling using a human proteome microarray

The HuProt™ version 3.1 arrays (CDI Laboratories, Mayaguez, PR) comprised of ~19,500 unique full-length human proteins were printed in duplicates, encompassing 81% of the proteome. Recombinant proteins were expressed in the yeast *S. cerevisiae*, purified, and printed on glass slides in duplicate, along with positive control proteins (H1, H2A, H2B, H3, H4, IgG488/594, Rhodamine + IgG 647, hMDM2, Era along with Human

IgG and Anti-Human IgG in various concentrations) and negative control proteins (BSA, Bovine serum albumin, buffer and GST in various concentrations) spotted in duplicates on the microarrays as well. This array has been previously utilized in profiling autoantibodies in human serum and tissue samples.³⁵⁻³⁷ Briefly, the HuProt array was blocked with 5% BSA/1×TBS-T at room temperature for 2h, and then incubated with 1:500 diluted serum samples in blocking buffer overnight at 4°C. The arrays were then washed with 1×TBS-T for 3 times, 10 min each, and probed with a cocktail of secondary antibody, containing 1:1000 dilution of fragment goat anti-human IgG conjugated with Cy3 (Jackson ImmunoResearch, West Grove, PA) and 1:1000 dilution of fragment goat anti-human IgM conjugated with Alexa Fluor® 647 (Jackson ImmunoResearch, West Grove, PA) for 1.5h at room temperature, followed by 3 washes (1×TBS-T, 10 min each) and spun to dryness prior to scanning.

Array data processing was performed by scanning the slides using a GenePix 4000B instrument (Molecular Dynamics, Sunnyvale, CA) and GenePix Pro (v7.2.22) software. Before pre-processing, the signal intensities for IgG binding to array features as well as any background signal present were measured and manually flagged. For each sample array, resulting GPR files were processed using the Bioconductor (v3.5) package PAA with modifications. Following pre-processing, the technical artifacts of any region and the overall quality of the individual arrays were confirmed by plotting foreground and background signal intensities against array position. The signal intensities of manually flagged probes were replaced by the median signal intensity for all unflagged probes, and arrays were subsequently corrected for background intensities using the Bioconductor package limma (v3.32.5) with the “normexp” model and a saddle-point

approximation. Of different normalization procedure such as cyclic loess, quantile, and VSN of the MA plots, the best normalization obtained with VSN was used. Finally, a combined signal intensity was generated from the duplicate probes for each antibody using the mean of the individual signal intensities and changing to log2 scale.

The candidate proteins involved in cardiac disease were prioritized using Phenolyzer (<http://phenolyzer.wglab.org/>), a tool that uses pre-compiled information and rank score to implicate proteins involved in cardiac diseases. The data interpretation uses the following criteria based on the ratio of signal intensity of the test groups with mean signal intensity of N_{UV}:

Class	COV_{UV} vs N_{UV} Fold Change (FC)	Cl_S vs N_{UV} Fold Change (FC)
Common	>2	>2
COVID-specific	>4	<1.5
Cl _S -specific	<1.5	>4

T/B/NK-lymphocyte sub-setting

Peripheral blood from the patient of interest and age- and vaccine type- matched naïve positive control was used for T-, B-, and NK- lymphocyte cell analysis. Enumeration of these T-, B-, and NK- lymphocytes was performed using an IVD-approved lymphocyte sub-setting analysis kit followed by flow cytometric (FC) analysis per the manufacturer's instructions (Becton Dickinson (BD)). Additional markers for extended lymphocyte sub-setting were also assessed on a BD FACSCanto-II 8-color instrument and data analyzed with BD FACSDiva version 8.0.1 analysis software. In addition, a basic immunophenotypic analysis was also performed on B lymphocytes, T-lymphocytes, NK

lymphocytes and plasma cells to assess for aberrancy on a BD FACSCanto 10-color instrument using CYTOPAINT CLASSIC version 1.2.0 analysis software.